A MULTIVARIATE ANALYSIS OF CERTAIN BIOCHEMICAL COMPONENTS OF EQUINE AND FELINE SERUM SAMPLES AS REPORTED BY AN AUTO-ANALYZER SYSTEM

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THESIS

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ABSTRACT

This thesis contains a multivariate statistical analysis of the results of an automated analysis of serum samples from the horse and cat. In the horse, 12 biochemical components plus body weight and age are recorded; thus, observations are made on 14 random variables. In the case of the cat there are observations on 13 random variables. Ninety-one (91) pair-wise correlation coefficients are computed from the equine data and 78 pair-wise correlation coefficients are computed from the feline data. Extensive hypothesis testing concerning these correlation coefficients is conducted and the results are presented. A discriminant analysis for 2 groups, male and female, is conducted for each species. In this analysis the vector of sample means of the biochemical components plus body weight and age for males is contrasted with the corresponding vector from females. Tolerance limits for each biochemical component measured are presented for both species.

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I. INTRODUCTION

There is a basic oneness of the human and veterinary medical professions [1]. Many diagnostic and therapeutic devices developed for use in one of these professions have sooner or later found equally significant application in the other. In particular, the advent of automated devices for the analysis of human serum has greatly facilitated biochemical profiling [2]. However, as Coffman reports, restricted availability of these devices in the past has limited their practicality in clinical veterinary medicine [3]. Coffman goes on to point out, and this writer has encountered no statement to the contrary, that the applicability of these devices, in enhancing the diagnostic capabilities of the clinician, will be cause for their increased utilization by the veterinary medical profession [4]. It is therefore logical to assume that automated biochemical profiling will be of increasing value in describing the normal intervals about the true biochemical parameters in the serum of domestic animals.

Although the auto-analyzer devices are capable of generating a large volume of data concerning biochemical values in human and animal sera, the proper analysis of these data should not be treated superficially. The lack of proficiency in statistical methodology by members of the bio-medical professions is an unfortunate fact [5].

Additionally, qualified statisticians are often asked to make meaningful conclusions from biological data that have been generated in meaningless fashion [6]. The increased reliance of the veterinary medical profession upon automated analyzing devices will yield little addition to our knowledge of serum biochemical parameters and their concomitant physiological significance unless the data are meaningfully collected and analyzed by sophisticated statistical techniques. It is this conclusion which has motivated the research effort reported herein.

II. A BRIEF CONSIDERATION OF THE AUTOANALYZER

The autoanalyzer used in this study was a Technicon Sequential Multiple Analyzer, SMA 12/60, which was first introduced in 1967 as a successor to the widely used SMA 12 [7]. The SMA 12/60 analyzes a serum sample quantitatively and reports on the concentration of 12 of the serum biochemical components on an easily interpreted pre-calibrated chart paper. The 12 biochemical components quantitatively analyzed are: calcium, inorganic phosphate, glucose, blood urea nitrogen (BUN), uric acid, cholesterol, total protein, albumen, total bilirubin, alkaline phosphatase, lactic dehydrogenase (LDH), and glutamic oxalacetic transaminase (SGOT).

The primary value of the autoanalyzer is that it provides a reasonable biochemical profile of the subject from a single serum sample within 60 seconds; it is invaluable as a screening device in that certain metabolic aberrations, whether suspected or unsuspected, will be manifested in abnormal serum chemistry charts.

III. THE MAMMALIAN SPECIES UNDER STUDY AND COLLECTION OF SAMPLES

The horse has recently enjoyed a resurgence of popularity although it is more in demand as a pleasure and recreation animal rather than a source of agricultural power. It was selected for this study because it was felt that the economic value of the horse justifies the most sophisticated diagnostic techniques by consulted veterinarians. While some data are available as to equine biochemical parameters the work thus far reported does not fully exploit the statistical potential of these data [8].

In this study, 44 horses of various breeds, 23 males and 21 females, were selected. All were clinically in good health. Blood samples were obtained by vena puncture; the serum was separated from the clot and was then centrifuged, frozen, and maintained in a frozen state until submitted to the laboratory.

The cat was selected for this study because information concerning biochemical parameters in the serum of this animal is particularly lacking [9]. Thirty (30) cats, 15 males and 15 females, all of mixed breed but of varying ages were included in this study. All were free of clinical illness. Blood samples were obtained by cardiac puncture and the samples were then processed as described above.

IV. TABULAR PRESENTATION OF DATA

In consideration of the equine data 14 variables were under consideration; in addition to the 12 serum biochemical components reported by the autoanalyzer, body weight and age were also considered. A previous report indicates that equine serum albumen is not reliably reported by the autoanalyzer [10]. Albumen is nonetheless included in this study to form a basis for substantiation or repudiation of this fact as may be justified by further research.

A similar approach was employed with the feline data with the exception that 13 variables were under consideration; inasmuch as no cat demonstrated measurable total bilirubin it was deleted from consideration.

The data as reported by the SMA 12/60 Auto-analyzer for the 44 horses and 30 cats are presented in Table I and Table II respectively.

TABLE I

SERUM BIOCHEMICAL VALUES IN 44 HORSES
AS REPORTED BY AN AUTO-ANALYZER SYSTEM

No.	Breed	Calcium	Inor. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	T. Bilirubin	Alkaline Phos.	LDH	SGOT	Weight	Age	Sex
1	Grade	11.2	3.3	86	10	0.5	110	6.4	0.6	0.9	87	211	460	850	13	F
2	Grade	11.8	3.1	85	11	0.5	135	6.9	0.6	1.0	105	224	560	1000	8	F
3	Qtr.Horse	12.0	6.4	98	13	0.5	135	5.7	0.6	0.7	490	250	650	500	0.5	M
4	Qtr.Horse	11.3	6.7	102	12	1.0	152	5.5	0.6	0.8	350	255	740	500	0.5	F
5	Qtr.Horse	11.2	4.0	76	16	0.4	101	6.5	0.5	1.3	111	205	560	1075	5	M*
6	Grade	10.5	3.1	79	19	0.4	94	6.8	0.4	2.0	118	187	1290	1100	14	F
7	Qtr.Paint	11.4	3.3	78	24	0.4	90	6.6	0.4	1.2	78	3.0	530	1050	9	M*
8	-	11.0	3.4	85	18	0.4	107	6.3	0.5	1.0	125	325	750	950	4	M*
9	-	12.1	3.7	94	21	0.4	142	6.6	0.5	1.0	172	259	560	1200	3	M*
10	-	11.6	3.7	83	14	0.7	112	6.5	0.6	0.8	120	250	840	1100	5	M*
11	-	11.1	3.7	80	16	0.4	111	6.3	0.5	0.6	138	250	1330	1050	14	F
12	-	12.2	5.1	105	13	0.4	101	6.6	0.4	0.9	335	240	700	800	1.5	F
13	Palomino	11.8	3.2	79	11	0.3	100	5.8	0.5	1.0	95	250	630	1100	7	M*
14	Grade	12.2	3.7	81	16	0.4	118	6.3	0.5	0.8	121	150	630	1100	8	F
15	Grade	11.9	3.8	86	15	0.8	110	6.0	0.6	1.2	107	265	630	1200	5	F
16	Grade	11.6	2.7	85	17	0.3	104	6.4	0.5	0.8	146	141	590	1100	4	M*
17	Qtr.Horse	11.5	3.7	72	11	0.3	112	6.5	0.5	1.1	92	252	590	1150	10	F

TABLE I (Continued

No.	Breed	Calcium	Inor. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	T. Bilirubin	Alkaline Phos.	LDH	SGOT	Weight	Age	Sex
18	Qtr.Horse	11.3	5.2	84	14	0.5	120	6.1	0.5	1.1	207	550	750	1000	2	F
19	Grade	11.4	4.1	85	15	0.4	130	6.5	0.4	1.7	138	185	540	1100	9	F
20	Appaloosa	13.1	3.2	85	16	0.3	104	6.5	0.5	0.8	150	200	530	900	6	M*
21	Grade	12.8	3.5	84	11	0.5	90	6.2	0.5	0.5	135	230	1080	1000	9	M*
22	Palomino	12.7	3.3	75	12	0.5	108	6.1	0.5	0.8	147	235	1410	1000	9	F
23	Appaloosa	10.5	4.4	81	8	0.4	120	6.5	0.1	6.1	135	221	750	1200	3	M*
24	Grade	12.1	3.5	75	14	0.4	119	6.5	0.4	2.3	105	206	850	1050	7	M*
25	Grade	11.5	3.7	84	16	0.4	130	6.4	0.4	1.8	99	206	680	900	13	F
26	Amer. Sadl.Bred	11.8	2.3	89	16	0.7	121	6.0	0.6	1.2	75	240	2500	1000	6	F
27	Arabian	11.9	4.4	85	15	0.4	107	6.4	0.5	1.2	114	213	600	1000	4	M*
28	Grade	12.2	2.9	80	8	0.4	107	7.0	0.5	1.0	106	165	410	900	6	F
29	Palomino	11.8	2.9	80	12	0.4	93	6.6	0.4	1.8	145	176	480	850	12	M*
30	Qtr.Horse	11.0	2.2	145	19	0.5	118	7.0	0.4	2.8	88	109	490	1000	12	M*
31	Std.Bred	12.3	2.9	97	13	0.4	110	7.1	0.4	2.1	125	224	800	1100	10	M*
32	Grade	11.4	3.7	87	16	0.4	150	6.3	0.5	1.0	96	190	640	900	7.5	F
33	Grade	11.4	4.1	80	17	0.6	92	6.7	0.5	1.2	195	260	640	750	4	F
34	Grade	11.2	3.9	77	15	0.4	95	6.4	0.5	1.6	171	168	630	1000	12	M*
35	Throw-Quarter	12.1	3.5	95	18	0.3	93	6.5	0.5	1.3	136	285	2100	1000	5	M*

TABLE I (Continued)

No.	Breed	Calcium	Inor. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	T. Bilirubin	Alkaline Phos.	LDH	SGOT	Weight	Age	Sex
36	Grade	12.3	3.6	70	13	0.4	110	7.6	0.5	2.8	149	155	1800	950	5	F
37	Grade	12.7	2.5	85	9	0.4	122	6.7	0.5	1.6	88	209	530	1050	9	F
38	Qtr.Horse	13.1	4.0	90	10	0.8	115	6.9	0.6	1.9	143	238	500	750	9	M*
39	Qtr.Horse	12.6	2.8	87	17	0.4	105	7.1	0.6	1.2	94	184	640	1200	9	M*
40	Palomino	11.7	2.9	80	10	0.5	132	7.1	0.4	1.5	225	255	700	1200	7	M*
41	Qtr.Horse Type	12.0	2.6	113	10	0.4	104	6.8	0.4	1.4	117	155	5.0	1100	8	M*
42	Arabian	12.1	4.4	98	19	0.4	113	6.2	0.5	0.9	170	205	640	800	2	F
43	Grade	12.0	3.2	85	18	0.4	100	6.7	0.4	1.6	104	154	660	1100	8	M*
44	Grade Qtr.Horse	11.1	2.8	88	22	0.3	117	6.1	0.4	1.3	150	195	700	800	9	F

F=21
M=23
44

TABLE II

SERUM BIOCHEMICAL VALUES FOR 30 CATS
AS REPORTED BY AN AUTO-ANALYZER SYSTEM

No.	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alkaline Phos.	LDH	SGOT	Weight	Age	Sex	Comments
1	10.4	7.3	69	24	1.2	115	6.4	1.8	72	240	135	7	2	M	
2	9.8	6.8	58	23	0.6	134	6.7	1.6	45	265	80	10	4	M	
3	9.5	7.8	70	26	0.7	76	5.5	1.2	87	3 5	70	4	0.5	M	
4	10.0	6.8	64	15	0.6	75	6.5	1.7	97	135	60	7	1	F	
5	10.1	7.3	54	26	0.8	75	6.4	1.6	62	195	62	8	1.5	M	
6	9.9	6.8	60	22	0.8	85	6.6	1.7	55	280	58	7	1	F	
7	9.2	6.0	60	23	0.9	107	7.7	1.5	30	375	85	12	3	F	
8	11.0	9.0	50	25	0.9	97	6.9	1.1	75	375	74	5	4	M	
9	9.6	8.7	68	27	1.0	112	7.1	1.5	97	310	100	5	4	F	
10	10.1	7.8	30	23	0.8	69	6.3	1.4	82	750	73	7	0.7	M	
11	10.2	8.0	69	26	1.0	95	6.3	1.5	60	310	48	7	2	M	
12	9.4	7.9	58	27	1.2	66	6.5	1.5	70	250	52	5	0.8	F	
13	9.6	7.9	80	28	1.2	45	6.2	1.4	140	320	72	3	0.3	M	
14	9.7	7.4	98	26	1.0	53	6.4	1.6	145	215	58	3	0.3	F	
15	9.1	5.9	65	41	0.9	152	7.1	1.7	41	220	78	7	3	F	Spayed
16	9.6	8.0	62	21	0.7	100	7.4	1.8	43	275	164	8	2	M	
17	9.3	8.5	38	21	0.7	131	6.6	1.6	82	340	93	7	1.5	M	

TABLE II (Continued)

No.	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alkaline Phos.	LDH	SGOT	Weight	Age	Sex	Comments
18	9.0	7.8	55	20	0.8	115	7.1	1.3	20	185	103	6	1	F	
19	9.6	8.2	112	27	0.8	146	6.5	1.8	60	335	177	11	2	M	
20	8.7	5.3	69	26	0.9	38	6.2	1.4	30	368	72	5	2	F	
21	8.5	6.1	55	34	1.1	26	7.7	1.4	26	216	103	6	2	F	
22	8.3	6.4	70	32	1.0	15	7.0	1.7	32	260	72	10	5	M	
23	8.3	7.4	90	29	1.0	100	7.0	1.5	31	251	90	8	6	M	
24	7.9	4.7	80	31	1.0	63	7.0	1.3	30	132	70	8	2	M	
25	10.0	9.2	110	26	0.9	69	7.0	1.6	62	299	152	5	0.6	F	
26	8.2	5.7	166	21	0.8	55	6.8	1.6	52	176	67	9	6	M	Neutered
27	8.1	5.8	117	26	0.9	51	7.3	1.8	57	77	63	12	5	F	
28	8.0	6.6	97	22	1.0	31	7.8	1.6	52	75	72	7	8	F	
29	8.3	8.4	95	18	0.9	30	6.3	1.3	56	290	83	5	1	F	
30	8.7	7.4	97	29	1.0	62	7.0	1.4	29	267	105	9	1.5	F	Spayed

V. SAMPLE STATISTICS

The analysis of the data is amenable to multivariate methodology. In the case of the equine data we have observations on 14 random variables:

$$X_{i,j} \qquad i = 1,2,\ldots\ldots,44$$
$$\qquad\quad j = 1,2,\ldots\ldots,14$$

where "i" refers to the identification of the animal and j refers to a variable.

In particular we have:

Random Variable	Definition
$X_{i,1}$	Calcium Level of Horse Number "i"
$X_{i,2}$	Inorganic Phosphate Level of Horse "i"
$X_{i,3}$	Glucose Level of Horse "i"
$X_{i,4}$	BUN Level of Horse "i"
$X_{i,5}$	Uric Acid Level of Horse "i"
$X_{i,6}$	Cholesterol Level of Horse "i"
$X_{i,7}$	Total Protein Level of Horse "i"
$X_{i,8}$	Albumen Level in Horse "i"
$X_{i,9}$	Total Bilirubin Level of Horse "i"
$X_{i,10}$	Alkaline Phosphatase Level of Horse "i"
$X_{i,11}$	LDH Level of Horse "i"
$X_{i,12}$	SGOT Level of Horse "i"
$X_{i,13}$	Body Weight of Horse "i"
$X_{1,14}$	Age of Horse "i"

In the case of the cat we have observations on 13 random variables:

$$Y_{i,j} \qquad i = 1,2,\ldots.,30$$
$$\qquad\qquad j = 1,2,\ldots.,13$$

where "i" and "j" again refer to the identification of the animal and the characteristic respectively.

In particular we have:

Random Variable	Definition
$Y_{i,1}$	Calcium Level of Cat "i"
$Y_{i,2}$	Inorganic Phosphate Level of Cat "i"
$Y_{i,3}$	Glucose Level of Cat "i"
$Y_{i,4}$	BUN Level of Cat "i"
$Y_{i,5}$	Uric Acid Level of Cat "i"
$Y_{i,6}$	Cholesterol Level of Cat "i"
$Y_{i,7}$	Total Protein Level of Cat "i"
$Y_{i,8}$	Albumen Level of Cat "i"
$Y_{i,9}$	Alkaline Phosphatase Level of Cat "i"
$Y_{i,10}$	LDH Level of Cat "i"
$Y_{i,11}$	SGOT Level of Cat "i"
$Y_{i,12}$	Body Weight of Cat "i"
$Y_{i,13}$	Age of Cat "i"

In the description of sample statistics that follow, the computational formulae are given for $x_{i,j}$. The same formulae apply for $y_{i,j}$ and it is understood that N = 44 and N = 30 for the horse and cat respectively.

In multivariate terminology, each horse may be represented as a 14 dimensional vector $\underline{x}^{(i)}$. For example, referring to Table I we see that horse number 1 is represented as follows:

$$\underline{x}^{(1)} = \begin{bmatrix} x_{1,1} \\ x_{1,2} \\ 3_{1,3} \\ x_{1,4} \\ x_{1,5} \\ x_{1,6} \\ x_{1,7} \\ x_{1,8} \\ x_{1,9} \\ x_{1,10} \\ x_{1,11} \\ x_{1,12} \\ x_{1,13} \\ x_{1,14} \end{bmatrix} = \begin{bmatrix} 11.2 \\ 3.3 \\ 86. \\ 10. \\ 0.5 \\ 110. \\ 6.4 \\ 0.6 \\ 0.9 \\ 87. \\ 211. \\ 460. \\ 850. \\ 13. \end{bmatrix}$$

The sample mean, $\bar{x}_{\cdot j}$, is required for each of the set of observations on the 14 variables and is computed as follows:

$$\bar{x}_{\cdot j} = \sum_{i=1}^{N} \frac{x_{i,j}}{N}$$

The sample means of the observations on the j variables may be represented as a j-dimensional sample mean vector, $\underline{\bar{x}}$, where

$$\bar{\underline{x}} = \begin{bmatrix} \bar{x}_{\cdot 1} \\ \bar{x}_{\cdot 2} \\ \bar{x}_{\cdot 3} \\ \vdots \\ \bar{x}_{\cdot j} \\ \bar{x}_{\cdot p} \end{bmatrix} = \frac{1}{N} \begin{bmatrix} \sum_{i=1}^{N} x_{1,1} \\ \sum_{i=1}^{N} x_{i,2} \\ \sum_{i=1}^{N} x_{i,3} \\ \vdots \\ \sum_{i=1}^{N} x_{i,j} \\ \vdots \\ \sum_{i=1}^{N} x_{i,p} \end{bmatrix}$$

The 6 sample mean vectors representing 44 horses, 23 male horses, 21 female horses, 30 cats, 15 male cats, 15 female cats, are presented in Table III.

The sample variance, s_j^2 or $s_{j,j}$ is computed for each set of observations on the j variables as follows:

$$s_j^2 = \frac{1}{N-1} \sum_{i=1}^{N} (x_{i,j} - \bar{x}_{\cdot j})^2$$

The sample standard deviation is computed by simply obtaining the square root of the sample variance:

$$s_j = \sqrt{s_j^2}$$

The sample covariance, $s_{j,k}$, between the observations $x_{i,j}$ and $x_{i,k}$, $i = 1,\ldots,N$, is computed as follows:

TABLE III

TABLE OF SAMPLE MEAN VECTORS

	44 Horses	23 Male Horses	21 Female Horses	30 Cats	15 Male Cats	15 Female Cats
CALCIUM (mg%)	11.78	11.87	11.70	9.27	9.46	9.08
INORGANIC PHOSPHATE (mg%)	3.62	3.49	3.75	7.23	7.39	7.07
GLUCOSE (mg%)	86.77	88.57	84.81	75.53	73.20	77.87
BUN (mg%)	14.55	14.78	14.29	25.50	25.53	25.46
URIC ACID (mg%)	0.45	0.43	0.48	0.90	0.88	0.93
CHOLESTEROL (mg%)	112.70	108.52	117.29	79.60 *91.79 **97.33	87.73	71.47
TOTAL PROTEIN (gms%)	5.49	6.55	6.43	6.78	6.60	6.95
ALBUMEN (gms%)	0.48	0.47	0.50	1.53	1.53	1.53
TOTAL BILIRUBIN	1.40	1.56	1.22	N/A	N/A	N/A
ALKALINE PHOSPHATASE T.U.	145.39	144.52	146.33	60.67	63.53	57.80
LDH T.U.	223.57	217.74	229.95	271.03	302.60	239.47
SGOT T.U.	786.36 636.05	714.35 **651.36	865.24 ***615.00	86.37	89.60	82.93
BODY WEIGHT	986.93	1022.83	947.62	7.10	7.47	6.73
AGE	7.14	6.89	7.40	2.26	2.43	2.08

Horses:

*6 animals with SGOT > 1200 T.U. deleted

**1 male with SGOT >1200 T.U. deleted

***5 females with SGOT > 1200 T.U. deleted

Cats:

*6 animals with cholesterol < 50 mg% deleted

**9 animals with cholesterol < 60 mg% deleted

$$s_{j,k} = \frac{1}{N-1} \sum_{i=1}^{N} (x_{i,j} - \bar{x}_{\cdot j})(x_{i,k} - \bar{x}_{\cdot k})$$

In multivariate analysis the sample variances of the observation on the variables and the sample covariances between each pair of these variables is presented as a sample variance-covariance matrix (S) with dimensions p by p. The elements of the sample variance-covariance matrix are as follows:

$$(S) = \begin{bmatrix} s_{1,1} & s_{1,2} & s_{1,3} & \cdots & s_{1,k} & \cdots\cdots & s_{1,p} \\ s_{2,1} & s_{2,2} & s_{2,3} & \cdots & s_{2,k} & \cdots\cdots & s_{2,p} \\ \vdots & & & & & & \\ s_{j,1} & s_{j,2} & s_{j,3} & \cdots & s_{j,k} & \cdots\cdots & s_{j,p} \\ \vdots & & & & & & \\ s_{p,1} & s_{p,2} & s_{p,3} & \cdots & s_{p,k} & \cdots\cdots & s_{p,p} \end{bmatrix}$$

In the main diagonal of the matrix j is equal to k and the main diagonal elements represent the sample variances for each of the j variables. Since $s_{j,k} = s_{k,j}$, for the sample variance-covariance matrices computed, those elements above the main diagonal will be deleted and the matrices will be lower triangular. The variance-covariance matrices for 44 horses, 23 male horses, 21 female horses, 30 cats, 15 male cats, and 15 female cats are presented in Tables IV, A thru F.

TABLE IV-A

VARIANCE-COVARIANCE MATRIX FOR 44 HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.377													
Inorganic Phosphate	-0.064	0.847												
Glucose	-0.134	0.162	155.388											
BUN	-0.580	-0.344	6.313	13.835										
Uric Acid	0.003	0.050	0.331	-0.112	0.021									
Cholesterol	-0.851	4.647	38.768	-7.533	0.716	234.304								
Total Protein	0.044	-0.184	0.542	-0.122	-0.015	-1.300	0.162							
Albumen	0.020	0.012	-0.041	-0.006	0.006	0.183	-0.009	0.008						
Total Bilirubin	-0.168	-0.067	0.488	-0.635	-0.012	0.437	0.129	-0.060	0.807					
Alkaline Phosphatase	1.599	54.875	209.320	-32.285	3.025	343.766	-10.681	0.854	-11.819	5988.949				
LDH	-4.670	25.102	-143.542	6.288	2.110	72.846	-9.280	0.976	-11.526	1189.516	4557.012			
SGOT	11.128	-45.801	-709.448	180.401	5.459	-765.981	-8.955	4.374	14.861	-2036.921	3396.754	188153.562		
Body Weight	-6.608	-84.671	-473.386	31.712	-8.340	-501.623	23.397	-4.545	36.570	-8091.316	-611.006	3887.413	26874.602	
Age	-0.268	-1.934	-4.317	0.250	-0.120	-13.191	0.430	-0.043	0.204	-153.134	-87.835	-21.936	161.124	12.888

TABLE IV-B

VARIANCE-COVARIANCE MATRIX FOR 23 MALE HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.432													
Inorganic Phosphate	-0.019	0.718												
Glucose	-0.466	-3.254	227.983											
BUN	-0.322	-0.588	7.583	15.632										
Uric Acid	0.014	0.023	0.237	-0.138	0.014									
Cholesterol	-0.472	4.078	52.419	-7.063	0.502	199.624								
Total Protein	0.028	-0.187	1.642	0.107	0.009	0.440	0.131							
Albumen	0.036	0.017	-0.048	0.092	0.004	-0.049	-0.008	0.012						
Total Bilirubin	-0.352	0.024	1.932	-1.509	0.003	3.262	0.106	-0.100	1.284					
Alkaline Phosphatase	4.196	49.804	49.463	-69.791	1.506	605.258	-12.615	2.164	-15.492	6708.227				
LDH	0.186	16.279	-317.527	26.213	0.663	73.870	-5.600	0.868	-11.520	701.460	2866.743			
SGOT	22.158	16.994	-216.205	48.716	-6.411	-1062.370	-13.782	1.476	-15,276	-359.644	6547.984	111907.188		
Body Weight	-27.692	-78.542	-308.941	69.960	-5.726	-95.404	25.573	-6.897	43.547	-8465.828	-867.636	2566.693	25733.664	
Age	0.253	-1.621	9.246	-0.206	0.042	-20.918	0.570	-0.290	0.193	-117.032	-70.348	-179.960	48.048	9.817

TABLE IV-C

VARIANCE-COVARIANCE MATRIX FOR 21 FEMALE HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.320													
Inorganic Phosphate	-0.092	0.994												
Glucose	-0.126	4.466	75.562											
BUN	-0.939	-0.021	4.207	12.414										
Uric Acid	-0.004	0.076	0.556	-0.074	0.029									
Cholesterol	-0.494	4.249	43.757	-6.036	0.746	242.014								
Total Protein	0.053	-0.174	-1.994	-0.414	-0.040	-2.684	0.195							
Albumen	0.006	0.002	0.035	-0.105	0.008	0.280	-0.008	0.005						
Total Bilirubin	-0.006	-0.123	-1.770	0.203	-0.019	-1.027	0.138	-0.013	0.261					
Alkaline Phosphatase	-1.008	62.936	399.365	7.850	4.797	64.599	-8.965	-0.580	-8.033	5495.410				
LDH	-9.105	34.312	65.840	-11.986	3.469	16.614	-12.964	0.910	-9.849	1773.714	6562.324			
SGOT	13.627	-138.788	-976.450	375.427	14.605	-1204.070	6.143	4.900	76.669	-4133.809	-911.236	268935.688		
Body Weight	9.238	-84.869	-832.974	-29.286	-9.548	-611.784	17.072	-0.750	16.809	-8009.137	144.879	11763.055	26369.012	
Age	-0.808	-2.452	-18.394	0.904	-0.319	-7.821	0.333	-0.070	0.322	-201.016	-114.905	108.273	314.760	16.765

TABLE IV-D

VARIANCE-COVARIANCE MATRIX FOR 30 CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.659												
Inorganic Phosphate	0.543	1.275											
Glucose	-10.159	-5.682	747.843										
BUN	-0.860	-1.591	-1.966	25.086									
Uric Acid	-0.022	-0.007	0.488	0.388	0.026								
Cholesterol	13.615	11.261	-269.951	2.793	-2.085	1342.660							
Total Protein	-0.171	-0.161	1.327	0.460	0.011	-0.289	0.265						
Albumen	0.003	-0.033	1.166	-0.022	-0.004	1.550	0.019	0.034					
Alkaline Phosphatase	12.210	15.090	-8.126	-30.207	0.456	-44.241	-8.098	0.069	952.366				
LDH	44.125	54.192	-1460.532	-30.431	-1.862	718.806	-21.410	-6.494	656.837	14758.688			
SGOT	3.215	13.244	113.074	-0.362	-0.353	443.633	3.957	1.782	-193.217	438.539	1046.445		
Body Weight	-0.556	-1.110	12.772	0.086	-0.104	19.628	0.533	0.211	-40.517	-42.383	12.859	5.610	
Age	-0.952	-1.065	23.093	0.867	0.006	-9.518	0.516	0.1193	-26.691	-99.122	-6.246	2.587	3.789

TABLE IV-E

VARIANCE-COVARIANCE MATRIX FOR 15 MALE CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.824												
Inorganic Phosphate	0.744	1.233											
Glucose	-15.777	-16.476	1065.456										
BUN	-1.270	-1.328	6.600	12.124									
Uric Acid	-0.008	-0.027	0.711	0.347	0.033								
Cholesterol	13.381	18.082	-173.514	-57.919	-2.463	1288.636							
Total Protein	-0.146	-0.136	1.793	0.029	-0.001	0.093	0.207						
Albumen	-0.022	-0.018	1.516	-0.158	-0.002	2.150	0.031	0.046					
Alkaline Phosphatase	12.430	16.322	-187.828	-18.948	1.176	-69.490	-8.436	-1.908	813.265				
LDH	56.811	80.101	-2246.338	-127.772	-4.073	241.028	-18.686	-6.739	1597.511	19689.227			
SGOT	3.870	11.290	139.471	-30.100	-0.733	760.941	4.886	5.170	-132.386	-77.513	1446.312		
Body Weight	-0.723	-0.965	21.971	0.233	-0.126	18.776	0.514	0.315	-48.195	-69.514	29.243	4.838	
Age	-1.099	-1.070	35.586	0.788	-0.007	-8.390	0.451	0.148	-36.933	-113.064	-3.821	2.626	3.608

TABLE IV-F

VARIANCE-COVARIANCE MATRIX FOR 15 FEMALE CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight	Age
Calcium	0.465												
Inorganic Phosphate	0.316	1.355											
Glucose	-4.317	5.489	471.980										
BUN	-0.526	-1.980	-10.505	39.838									
Uric Acid	-0.029	0.019	0.182	0.458	0.019								
Cholesterol	11.510	2.513	-345.004	63.124	-1.449	1350.835							
Total Protein	-0.136	-0.139	0.072	0.938	0.016	2.388	0.276						
Albumen	0.029	-0.050	0.883	0.112	-0.006	1.119	0.007	0.024					
Alkaline Phosphatase	11.696	13.973	185.328	-43.828	-0.087	-72.114	-7.253	2.071	1141.884				
LDH	21.738	21.556	-621.218	62.481	1.794	697.765	-13.712	-6.488	-430.828	8747.109			
SGOT	1.392	14.991	111.919	29.105	0.173	98.176	4.611	-1.455	-288.942	753.675	696.065		
Body Weight	-0.577	-1.458	6.319	-0.081	-0.071	15.490	0.730	0.124	-37.986	-43.081	-5.305	6.495	
Age	-0.944	-1.195	13.133	0.996	0.028	14.404	0.684	0.100	-19.440	-104.211	-10.416	2.594	4.175

The primary purpose of computing the sample variance-covariance matrix is in computing the Pearson Product Moment Correlation Coefficient, $r_{j,k}$. This statistic is a measure of the tendency of 2 variables to vary together in linear fashion. It is described as the ratio of the sample covariance between 2 variables to the square root of the product of the sample variances of the 2 variables; that is,

$$r_{j,k} = \frac{s_{j,k}}{\sqrt{(s_{j,j})(s_{k,k})}}$$

For the equine data there are $\binom{14}{2} = 91$ pair-wise correlation coefficients to be computed and for the feline data there are $\binom{13}{2} = 78$ pair-wise correlation coefficients to compute and consider.

The tests of hypotheses conducted in this research effort rely on certain assumptions concerning the sample statistics. The central limit theorem, which permits us to assume a normal $(\mu, \frac{\sigma^2}{N})$ distribution of sample means in the univariate case [11], also permits us to make the assumption of normality in the multivariate case; namely, the distribution of the sample mean vectors, $\underline{\bar{x}}$, is normal with mean $\underline{\mu}$ and variance-covariance matrix $\frac{1}{N} \Sigma$ [12]. This assumption will be employed in testing the notion that the vector of sample means of the males of a given species does not differ significantly from that of females of the same species.

Additionally, if the population correlation coefficient, $\rho_{j,k}$, between 2 variables equals zero, then the sample

correlation coefficient, $r_{j,k}$ is distributed as

$$\frac{t_{(N-2)}\sqrt{(1-r_{j,k}^2)}}{\sqrt{N-2}}$$

where $t_{(N-2)}$ represents the Student "t" distribution with N-2 degrees of freedom [13]. Equivalently, the statistic

$$\frac{r_{j,k}\sqrt{N-2}}{\sqrt{1-r_{j,k}^2}}$$

is distributed according to the Student "t" distribution with N-2 degrees of freedom, provided that the hypothesis that $\rho_{j,k} = 0$ is true.

VI. HYPOTHESES TESTED

A "null" hypothesis, denoted as H_o, can be defined as a statement or notion for which it is possible to compute a statistic and the corresponding probability of a more extreme value of that statistic. Alternatively, it is common to establish a probability corresponding to the risk of rejecting H_o when, in fact, it is true and denoting this probability as α. H_o is then rejected only when the test statistic is more extreme than that associated with α.

In this study the following null hypotheses were tested:

1. H_o: The true correlation coefficient ($\rho_{j,k}$) between variables j and k is equal to zero. This hypothesis was tested for both equine and feline data irrespective of the sex of the animal.

Since the sample correlation coefficient can be converted into a "$t_{(N-2)}$" statistic, providing H_o is true, it is possible to solve for the "critical" value of $r_{j,k}$ by establishing a type I error (α) and locating the appropriate "$t_{(N-2)}$" value from a statistical table. In fact, this critical value of $r_{j,k}$, denoted $r_{\alpha j,k}$, is obtained as follows:

$$r_{\alpha j,k} = \frac{t_\alpha}{\sqrt{t_\alpha^2 + (N-2)}}$$

where t_α is the $1-\alpha$ percentile point of the "t" distribution with N-2 degrees of freedom. Conveniently, values of

$r_{\alpha j,k}$ are already tabulated [14]. Those appropriate to the testing of this null hypothesis are presented in Table V.

TABLE V

CRITICAL VALUES OF $r_{j,k}$ FOR SPECIFIED α

N	$\alpha = 0.05$	$\alpha = 0.01$
44 (Horses)	0.298	0.385
30 (Cats)	0.367	0.470

Triangular matrices of sample correlation coefficients for the 44 horses and 30 cats are presented in Tables VI-A and VI-B respectively. In these tables those values of $r_{j,k}$ for which H_o: $\rho_{j,k} = 0$ can be rejected at $\alpha = 0.05$ are indicated by a* and those for which the hypothesis can be rejected at $\alpha = 0.01$ are indicated with **.

TABLE VI-A
CORRELATION MATRIX - 44 HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight
Calcium													
Inorganic Phosphate	-0.113												
Glucose	-0.018	0.014											
BUN	-0.254	-0.100	0.136										
Uric Acid	0.033	0.376 *	0.183	-0.207									
Cholesterol	-0.090	0.330 *	0.203	-0.132	0.322 *								
Total Protein	0.179	-0.499 **	0.011	-0.081	-0.264	-0.211							
Albumen	0.352 *	0.140	-0.036	-0.018	0.457 **	0.130	-0.242						
Total Bilirubin	-0.305 *	-0.081	0.044	-0.190	-0.089	0.032	0.358 *	-0.727 **					
Alkaline Phosphatase	0.034	0.770 **	0.217	-0.112	0.269	0.290	-0.343 *	0.120	-0.170				
LDH	-0.113	0.404 **	-0.171	0.025	0.215	0.070	-0.342 *	0.157	-0.190	0.228			
SGOT	0.042	-0.115	-0.131	0.112	0.087	-0.115	-0.051	0.109	0.038	-0.061	0.116		
Body Weight	-0.066	-0.561 **	-0.232	0.052	-0.350 *	-0.200	0.355 *	-0.301	0.248	-0.638 **	-0.055	0.055	
Age	-0.122	-0.586 **	-0.096	0.019	-0.231	-0.240	0.298 *	-0.129	0.063	-0.551 **	-0.362	-0.014	0.274

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geqq 0.298$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geqq 0.385$)

TABLE VI-B

CORRELATION MATRIX - 30 CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight
Calcium												
Inorganic Phosphate	0.592 **											
Glucose	-0.458 *	-0.184										
BUN	-0.212	-0.281	-0.014									
Uric Acid	-0.171	-0.040	0.110	0.482 **								
Cholesterol	0.458 *	0.272	-0.269	0.015	-0.354							
Total Protein	-0.408 *	-0.278	0.094	0.178	0.134	-0.015						
Albumen	0.020	-0.159	0.232	-0.024	-0.120	0.230	0.204					
Alkaline Phosphatase	0.487 **	0.433 *	-0.010	-0.195	0.092	-0.039	-0.509 **	0.012				
LDH	0.447 *	0.395 *	-0.440 *	-0.050	-0.095	0.162	-0.342	-0.290	0.175			
SGOT	0.122	0.363	0.128	-0.002	-0.068	0.374 *	0.238	0.299	-0.194	0.112		
Body Weight	-0.289	-0.415 *	0.197	0.007	-0.272	0.226	0.437 *	0.483 **	-0.554 **	-0.147	0.168	
Age	-0.602 **	-0.484 **	0.434 *	0.089	0.018	-0.133	0.514 **	0.333	-0.444 *	-0.419 *	-0.099	0.561 **

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geq 0.367$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geq 0.470$)

2. H_o: The true coefficient of correlation, $\rho^m_{j,k}$, between variables j and k for the males is zero. This hypothesis is tested separately for horses and cats. The critical values of $r_{j,k}$ are presented in Table VII.

TABLE VII

CRITICAL VALUE OF $r_{j,k}$ FOR THE SPECIFIED α

N	$\alpha = 0.05$	$\alpha = 0.01$
23 (Male Horses)	0.413	0.526
15 (Male Cats)	0.514	0.641

Triangular matrices of sample correlation coefficients for the 23 male horses and 15 male cats are presented in Tables VIII-A and VIII-B respectively. Significant values are indicated as previously described.

TABLE VIII-A
CORRELATION MATRIX - MALE HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight
Calcium													
Inorganic Phosphate	-0.035												
Glucose	-0.047	-0.254											
BUN	-0.124	-0.176	0.127										
Uric Acid	0.178	0.229	0.132	-0.296									
Cholesterol	-0.051	0.341	0.246	-0.126	0.300								
Total Protein	0.119	-0.610 **	0.301	0.075	0.216	0.086							
Albumen	0.512 *	0.187	-0.030	0.218	0.302	-0.032	-0.209						
Total Bilirubin	-0.472 *	0.025	0.113	-0.337	0.023	0.204	0.259	-0.825 **					
Alkaline Phosphatase	0.078	0.718 **	0.040	-0.216	0.155	0.523 *	-0.426 *	0.247	-0.167				
LDH	0.005	0.359	-0.393	0.124	0.104	0.098	-0.289	0.151	-0.190	0.160			
SGOT	0.101	0.060	-0.043	0.037	-0.162	-0.225	-0.114	0.041	-0.040	-0.013	0.366		
Body Weight	-0.262	-0.578 **	-0.128	0.110	-0.301	-0.042	0.441 *	-0.402	0.240	-0.644 **	-0.101	0.048	
Age	0.123	-0.611 **	0.195	-0.017	0.113	-0.472 *	0.503 *	-0.086	0.054	-0.456 *	-0.419 *	-0.172	0.096

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geq 0.413$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geq 0.526$)

TABLE VIII-B

CORRELATION MATRIX - MALE CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight
Calcium												
Inorganic Phosphate	0.739 **											
Glucose	-0.532 *	-0.455										
BUN	-0.402	-0.344	0.058									
Uric Acid	-0.048	-0.132	0.120	0.548 *								
Cholesterol	0.411	0.454	-0.148	-0.463	-0.377							
Total Protein	-0.353	-0.269	0.121	0.018	-0.017	0.006						
Albumen	-0.111	-0.073	0.216	-0.211	-0.040	0.278	0.321					
Alkaline Phosphatase	0.480	0.516 *	-0.202	-0.191	0.226	-0.068	-0.650 **	-0.311				
LDH	0.446	0.514 *	-0.490	-0.262	-0.159	0.048	-0.293	-0.223	0.399			
SGOT	0.112	0.267	0.112	-0.227	-0.106	0.557 *	0.282	0.631 *	-0.122	-0.014		
Body Weight	-0.362	-0.395	0.306	0.030	-0.314	0.238	0.514 *	0.666 **	-0.768 **	-0.225	0.350	
Age	-0.638 *	-0.508	0.574 *	0.119	-0.021	-0.123	0.522 *	0.363	-0.682 **	-0.424	-0.053	0.629 *

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geqq 0.514$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geqq 0.641$)

3. H_o: The true correlation coefficient, $\rho^f_{j,k}$, between variables j and k for females is equal to zero. This hypothesis is tested separately for horses and cats. The critical values of $r_{j,k}$ are specified in Table IX.

TABLE IX

CRITICAL VALUE OF $r_{j,k}$ FOR THE SPECIFIED α

N	$\alpha = 0.05$	$\alpha = 0.01$
21 (Female Horses)	0.433	0.549
15 (Female Cats)	0.514	0.641

Triangular matrices of sample correlation coefficients for the 21 female horses and 15 female cats are presented in Tables X-A and X-B. Significance is indicated as previously specified.

TABLE X-A
CORRELATION MATRIX - FEMALE HORSES

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles-terol	Total Protein	Albumen	Total Bilirubin	Alka. Phos.	LDH	SGOT	Body Weight
Calcium													
Inorganic Phosphate	-0.163												
Glucose	-0.026	0.515 *											
BUN	-0.470 *	-0.006	0.137										
Uric Acid	-0.043	0.448 *	0.378	-0.125									
Cholesterol	-0.056	0.274	0.324	-0.110	0.283								
Total Protein	0.210	-0.394	-0.506 *	-0.266	-0.534 *	-0.390							
Albumen	0.162	0.028	0.057	-0.421	0.669 **	0.254	-0.256						
Total Bilirubin	-0.022	-0.241	-0.399	0.113	-0.220	-0.129	0.613 **	-0.360					
Alkaline Phosphatase	-0.024	0.852 **	0.620 **	0.030	0.382	0.056	-0.274	-0.111	-0.212				
LDH	-0.198	0.425	0.094	-0.042	-0.253	0.013	-0.362	0.159	-0.238	0.295			
SGOT	0.046	-0.268	-0.217	0.206	0.166	-0.149	0.027	0.134	0.289	-0.108	-0.022		
Body Weight	0.100	-0.524 *	-0.590 **	-0.051	-0.348	-0.242	0.238	-0.065	0.203	-0.665 **	0.011	0.140	
Age	-0.349	-0.601 **	-0.517 *	0.063	-0.461 *	-0.123	0.184	-0.242	0.154	-0.662 **	-0.346	0.051	0.473 *

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geq 0.433$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geq 0.549$)

TABLE X-B

CORRELATION MATRIX - FEMALE CATS

	Calcium	Inorg. Phos.	Glucose	BUN	Uric Acid	Choles- terol	Total Protein	Albumen	Alka. Phos.	LDH	SGOT	Body Weight
Calcium												
Inorganic Phodphate	0.398											
Glucose	-0.292	0.217										
BUN	-0.122	-0.269	-0.077									
Uric Acid	-0.304	0.120	0.060	0.523 *								
Cholesterol	0.460	0.059	-0.432	0.272	-0.284							
Total Protein	-0.380	-0.228	0.006	0.283	0.214	0.124						
Albumen	0.278	-0.277	0.264	0.115	-0.278	0.197	0.091					
Alkaline Phosphatase	0.508	0.355	0.252	-0.206	-0.019	-0.058	-0.409	0.397				
LDH	0.341	0.198	0.306	0.106	0.138	0.203	-0.279	-0.450	-0.136			
SGOT	0.077	0.488	0.195	0.175	0.047	0.101	0.333	-0.357	-0.324	0.305		
Body Weight	-0.332	-0.491	0.114	-0.005	-0.201	0.165	0.545 *	0.315	-0.441	-0.181	-0.079	
Age	-0.678 **	-0.502	0.296	0.077	0.098	-0.192	0.638 *	0.317	-0.282	-0.545 *	-0.193	0.498

* - significant at $\alpha = 0.05$ ($|r_{j,k}| \geqq 0.514$); ** - significant at $\alpha = 0.01$ ($|r_{j,k}| \geqq 0.641$)

4. H_o: $\rho^m_{j,k} = \rho^f_{j,k} = \rho_{j,k}$

This hypothesis states that the sample correlation coefficients between variables j and k for males and females, considered as separate entities, are actually estimating a common population correlation coefficient, $\rho_{j,k}$ [15]. Alternatively, the hypothesis states that $\rho^m_{j,k} - \rho^f_{j,k} = 0$. Note that there is no assumption that $\rho_{j,k} = 0$.

To test this hypothesis Fisher's Z transformation is employed where

$$Z^m_{j,k} = \frac{1}{2} \ln \left[\frac{1 + r^m_{j,k}}{1 - r^m_{j,k}}\right]$$

and

$$Z^f_{j,k} = \frac{1}{2} \ln \left[\frac{1 + r^f_{j,k}}{1 - r^f_{j,k}}\right]$$

It will be noted that each $Z_{j,k}$ is the inverse hyperbolic tangent of $r_{j,k}$ [16]. If H_o is true, then

$$\frac{\left|Z^m_{j,k} - Z^f_{j,k}\right|}{\sqrt{\frac{1}{N^m-3} + \frac{1}{N^f-3}}}$$

has a standard normal distribution [17]. In the equine data, $N^m = 23$ and $N^f = 21$. The quantity $\sqrt{\frac{1}{N^m-3} + \frac{1}{N^f-3}}$ therefore becomes $\sqrt{\frac{1}{20} + \frac{1}{18}} \simeq \sqrt{0.10555...} \simeq 0.325$. To reject H_o at

the $\alpha = 0.05$ level $(|z^m_{j,k} - z^f_{j,k}|)$, must be greater than or equal to $(1.96)(0.325) = 0.637$. To reject H_o at the $\alpha = 0.01$ level this absolute difference must be greater than or equal to $(2.575)(0.325) = 0.837$. It should be recalled that 1.96 and 2.575 represent the $(1-\alpha) = 0.95$ and $(1-\alpha) = 0.99$ percentile points of the standard normal distribution, respectively.

In the equine data, statistically significant differences between male and female sample correlation coefficients are presented in Table XI.

TABLE XI

Paired Variables	$r^m_{j,k}$	$z^m_{j,k}$	$r^f_{j,k}$	$z^f_{j,k}$	$\|z^m_{j,k} - z^f_{j,k}\|$
Inorganic Phosphate & Glucose	-0.254	-0.260	0.515	0.570	0.830*
Glucose and Age	0.195	-0.198	-0.517	-0.572	0.770*
Uric Acid and Total Protein	0.216	0.220	-0.534	-0.596	0.816*
Glucose and Total Protein	0.301	0.311	-0.506	-0.557	0.868**

* - significant at $\alpha = 0.05$
** - significant at $\alpha = 0.01$

In the feline data there were no statistically significant differences between any of the male and female correlation coefficients. Therefore, H_o cannot be rejected in the case of the cat, at $\alpha = 0.05$.

5. H_o: $\underline{\mu}^m - \underline{\mu}^f = 0$; that is, the sample mean vector for males and the sample mean vector for females came from the same parent population with mean vector $\underline{\mu}$. To test the validity of H_o, Mahalanobis' D^2 statistic is computed as follows:

$$[\bar{\underline{x}}^m - \bar{\underline{x}}^f]^T \; S^{-1} [\bar{\underline{x}}^m - \bar{\underline{x}}^f] = D^2$$

where $[\bar{\underline{x}}^m - \bar{\underline{x}}^f]^T$ is the transpose of the 14 by 1 vector of the arithmetic difference between the male and female vectors of sample means, S^{-1} is the inverse of the pooled variance-covariance matrix S,

where $$S = \frac{(N^m-1)S^m + (N^f-1)S^f}{N^m + N^f - 2}$$

and S^m is the variance-covariance matrix for male data

S^f is the variance-covariance matrix for female data

N^m is the male sample size

N^f is the female sample size.

If H_o is true, then $D^2 \left[\dfrac{N^m N^f (N^m+N^f - p - 1)}{p(N^m+N^f)(N^m+N^f-2)}\right]$ is distributed in accordance with the F distribution that has (p, N^m+N^f-p-1) degrees of freedom [18]. The results of this hypothesis test are presented in Table XII.

TABLE XII

DISCRIMINANT ANALYSIS BETWEEN MALES AND FEMALES

Species	Computed D^2	Degrees of Freedom	Computed F Value	F at $\alpha = 0.05$
Equine	2.537	(14,29)	1.374	2.10
Feline	5.748	(13,16)	1.895	2.40

Thus, for each species, the computed F statistic is not significant at $\alpha = 0.05$ and H_o cannot be rejected at that level of significance.

VII. TOLERANCE LIMITS

Upper and lower tolerance levels based on the observed data are determined as follows: each $\bar{x}_{\cdot j}$ is estimating a population mean, $\mu_{\cdot j}$; each s_j^2 is estimating a population variance, σ_j^2. μ_j and σ_j^2 are fixed but unknown parameters. The statistics $\bar{x}_{\cdot j}$ and s_j^2 are random variables. It is possible to determine a constant, K, such that in a large series of samples from a normal distribution, a fixed proportion, γ, of the intervals $\bar{x}_{\cdot j} \pm K_\alpha\sqrt{s_j^2}$ will include 100 $(1-\alpha)$% or more of the distribution. Thus, statistical tolerance limits for a normal random variable, $X_{i,j}$, are given by the following:

$$L = \bar{x}_{\cdot j} - K_\alpha\sqrt{s_j^2} \quad \text{and} \quad U = \bar{x}_{\cdot j} + K_\alpha\sqrt{s_j^2}$$

These limits have the property that the probability that the interval includes at least a specified proportion $(1-\alpha)$ of the distribution is equal to a preassigned value α [19]. In this paper, values of 0.95 and 0.05 were assigned to γ and α respectively. The following $K_{0.05}$ values such that the probability is 0.95 that at least 95% of the distribution will be included between $\bar{x}_{\cdot j} \pm K_{0.05}\sqrt{s_j^2}$ are presented:

N	$K_{0.05}$
44	2.415
38	2.464
30	2.549
24	2.651
21	2.723

These values are employed in constructing the tolerance limits for the biochemical components of each species. These limits are presented in Tables XIII-A and XIII-B.

TABLE XIII-A

TOLERANCE LIMITS IN THE HORSE

Biochemical Component	N	$\bar{x}_{.j}$	S_j	$K_{.05}$	$\bar{x}_{.j} \pm K_{.05}\, S$
Calcium	44	11.78	0.61	2.415	10.31 - 13.25
Inorganic Phosphate	44	3.62	0.92	2.415	1.40 - 5.84
Glucose	44	86.77	12.47	2.415	56.65 - 116.89
BUN	44	14.55	3.72	2.415	5.57 - 23.53
Uric Acid	44	0.45	0.15	2.415	0.09 - 0.81
Cholesterol	44	112.70	15.31	2.415	75.73 - 149.67
Total Protein	44	6.49	0.40	2.415	5.52 - 7.46
Albumen	44	0.48	0.09	2.415	0.26 - 0.70
Total Bilirubin	44	1.40	0.90	2.415	0 - 3.57
Alkaline Phosphatase	44	145.39	77.39	2.415	0 - 332.29
LDH	44	223.57	67.51	2.415	60.53 - 386.61
SGOT	44	786.36	433.77	-	- - -
	38	636 05	127.80	2.464	321.15 - 950.95

TABLE XIII-B

TOLERANCE LIMITS IN THE CAT

Variable	N	$\bar{x}$	S	$K_{.05}$	KS	$\bar{x} \pm KS$
Calcium	30	9.27	0.81	2.549	2.06	7.21 - 11.33
Inorganic Phosphate	30	7.23	1.13	2.549	2.88	4.35 - 10.11
Glucose	30	78.53	27.35	2.549	69.72	8.81 - 148.25
BUN	30	25.50	5.01	2.549	12.77	12.73 - 38.27
Uric Acid	30	0.90	0.16	2.549	0.41	0.49 - 1.31
Choles-terol	30	79.60	36.64	2.549	93.40	0 - 173.00
	24	91.79	29.91	2.651	79.29	12.50 - 171.08
	21	97.33	27.76	2.723	75.59	21.74 - 172.92
Total Protein	30	6.78	0.52	2.549	1.33	5.45 - 8.11
Albumen	30	1.53	0.18	2.549	0.46	1.07 - 1.99
Alkaline Phos.	30	60.67	30.86	2.549	78.66	0 - 139.33
LDH	30	271.03	121.49	2.549	309.68	0 - 580.71
SGOT	30	86.37	32.35	2.549	82.46	3.91 - 168.83

VIII. SUMMARY

A summary of the significant findings of this study will be presented according to species. In this summary, "significant," as applied to sample correlation coefficients, refers to an ability to reject H_o: $\rho_{j,k} = 0$ at $\alpha \leqq 0.05$.

A. EQUINE DATA

In the case of the data pertaining to the 44 horses, significant positive correlation coefficients were obtained for the 23 males in the following pair-wise observa ions:

Calcium and Albumen

Inorganic Phosphate and Alkaline Phosphatase

Cholesterol and Alkaline Phosphatase

Total Protein and Body Weight

Total Protein and Age

Significant negative correlation coefficients for the 23 male horses were obtained in the following paired observations:

Calcium and Total Bilirubin

Inorganic Phosphate and Total Protein

Inorganic Phosphate and Body Weight

Inorganic Phosphate and Age

Cholesterol and Age

Total Protein and Alkaline Phosphatase

Albumen and Total Bilirubin

Alkaline Phosphatase and Body Weight

Alkaline Phosphatase and Age

LDH and Age

Significant positive correlation coefficients were observed in the sample of 21 female horses between the following paired variables:

*Inorganic Phosphate and Glucose

Inorganic Phosphate and Uric Acid

Inorganic Phosphate and Alkaline Phosphatase

Glucose and Alkaline Phosphatase

Uric Acid and Albumen

Total Protein and Total Bilirubin

Body Weight and Age

Significant negative correlation coefficients were observed in the sample of 21 female horses between the following paired variables:

Calcium and BUN

Inorganic Phosphate and Body Weight

Inorganic Phosphate and Age

Glucose and Body Weight

*Glucose and Age

*Uric Acid and Total Protein

Uric Acid and Age

Glucose and Total Protein

Alkaline Phosphatase and Body Weight

Alkaline Phosphatase and Age

Those correlation coefficients in the female found to be significantly different from the corresponding correlation coefficient in the male are indicated with a *.

Significant positive correlation coefficients in all 44 horses as a group that were not significant in either of the sub-groups considered separately were observed between the following paired variables:

Inorganic Phosphate and Cholesterol

Uric Acid and Cholesterol

Inorganic Phosphate and LDH

Significant positive correlation coefficients in all 44 horses as a group that were also significant in both the male and female sub-groups were observed between the following paired variables:

Inorganic Phosphate and Alkaline Phosphatase

Significant negative correlation coefficients in all 44 horses that were also significant in both the male and female sub-groups are as follows:

Inorganic Phosphate and Body Weight

Inorganic Phosphate and Age

Alkaline Phosphatase and Body Weight

Alkaline Phosphatase and Age

All other positive and negative correlation coefficients that were observed to be significant in all 44 horses appear to have reached significant levels only because of a very high correlation coefficient in one of the sub-groups.

In considering the fifth hypothesis, it is concluded from this study that there is no difference between male and female with respect to the mean values of the 12 serum biochemical parameters as reported by the autoanalyzer in the population of horses from which this sample of 44 horses is a representative sample.

B. FELINE DATA

With respect to 15 female cats, the following positive bivariate correlation coefficients were found to be significant:

BUN and Uric Acid

Total Protein and Body Weight

Total Protein and Age

The following negative bivariate correlation coefficients were found to be significant in the data obtained from the 15 female cats:

Calcium and Age

LDH and Age

The 15 male cats showed the following positive correlation coefficients that were statistically significant:

Calcium and Inorganic Phosphate

Inorganic Phosphate and Alkaline Phosphatase

Inorganic Phosphate and LDH

Glucose and Age

BUN and Uric Acid

Cholesterol and SGOT

Total Protein and Age

Albumen and Body Weight

Body Weight and Age

The following statistically significant bivariate correlation coefficients were demonstrated from the male data:

Calcium and Glucose

Total Protein and Alkaline Phosphatase

Alkaline Phosphatase and Body Weight

Alkaline Phosphatase and Age

None of the correlation coefficients in one sex differed significantly from the corresponding coefficient in the other sex. Admittedly, the relatively small size of the sub-groups is a limiting factor.

The influence of the number of observations on the significance of correlation coefficients is evidenced when we consider the 30 cats as a single group. Significant positive correlation coefficients in all 30 cats were obtained for the following paired variables:

Calcium and Inorganic Phosphate

*Calcium and Cholesterol

*Calcium and Alkaline Phosphatase

*Calcium and LDH

Inorganic Phosphate and Alkaline Phosphatase

Inorganic Phosphate and LDH

Glucose and Age

BUN and Uric Acid

Cholesterol and SGOT

*Total Protein and Body Weight

Total Protein and Age

Albumen and Body Weight

Body Weight and Age

The following significant negative correlation coefficients between paired variables were observed in the 30 cats:

Calcium and Glucose

*Calcium and Total Protein

Calcium and Age

*Inorganic Phosphate and Body Weight

*Inorganic Phosphate and Age

Glucose and LDH

Total Protein and Alkaline Phosphatase

Alkaline Phosphatase and Body Weight

Alkaline Phosphatase and Age

LDH and Age

Significant correlation coefficient in the combined sample of 30 cats that were not significant in either males or females considered separately are indicated with a *.

Certainly, it would appear that additional observations in both male and female cats could result in an increased number of bivariate correlation coefficients attaining significance; this is particularly true of the female group.

With respect to the fifth hypothesis of equality of the mean vectors, it is concluded that there is no difference between males and females in the means of the 12 biochemical components in the feline population from which this sample of 30 is representative.

IX. RECOMMENDATIONS

The analysis of the equine biochemical data suggests future work is desirable, particularly in the measurement of albumen, LDH, and SGOT as reported by the autoanalyzer. The significant bivariate correlation coefficients, particularly as they occur in the female, should be exploited for possible diagnostic significance.

The analysis of the feline biochemical data demonstrates that animals in apparent robust health can nonetheless be hypercholesterolemic, at least as reported by the autoanalyzer. Even when the 9 most extreme cases were ignored the lower limit of the cholesterol tolerance was considerably below those values previously reported by Cornelius and Kaneko [20].

This work has been undertaken as a pilot study in multivariate analysis of biochemical components of the serum of two of the common species of domestic animals. Emphasis has been placed on tolerance interval estimations, possibly significant bivariate linear association as measured by the Pearson Product Moment Correlation Coefficient, and a discriminant analysis of the male and female sample mean vectors. With the increased use of the autoanalyzer and increased availability of data, this method of statistical analysis can and should be expanded.

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13. ABSTRACT

This thesis contains a multivariate statistical analysis of the results of an automated analysis of serum samples from the horse and cat. In the horse, 12 biochemical components plus body weight and age are recorded; thus, observations are made on 14 random variables. In the case of the cat there are observations on 13 random variables. Ninety-one (91) pair-wise correlation coefficients are computed from the equine data and 78 pair-wise correlation coefficients are computed from the feline data. Extensive hypothesis testing concerning these correlation coefficients is conducted and the results are presented. A discriminant analysis for 2 groups, male and female, is conducted for each species. In this analysis the vector of sample means of the biochemical components plus body weight and age for males is contrasted with the corresponding vector from females. Tolerance limits for each biochemical component measured are presented for both species.

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